Institution: University of Manchester

Unit of Assessment: UoA5

Title of case study: Antibodies to ACTH and related hormones as diagnostic tools

1. Summary of the impact

Measurement of hormones is essential to the understanding and diagnosis of endocrine diseases. White and her research group have developed unique antibodies that are widely used in diagnostic assays for adrenocorticotrophic hormone (ACTH) and related peptides, including the first and only kit for measuring pro-opiomelanocortin (POMC), the precursor of ACTH. These assays are used worldwide for diagnosis, decisions on treatment, monitoring for recurrence of tumours and prognosis in a number of patient groups with life-threatening endocrine disorders. Global sales of the ACTH Elecsys tests by Roche exceeded 6 million kits since 2008. AstraZeneca has used the POMC and ACTH assays in its drug discovery programmes in the cardiovascular and metabolic diseases therapy area. The antibodies therefore have had health impact in relieving suffering and in improving patient care, as well as commercial impact in worldwide sales of assays and influencing drug development strategies.

2. Underpinning research

The impact is based on research at the University of Manchester from 1993 to the present. The key researchers were:

Professor Anne White, Professor of Endocrine Science (1981-present) - devised the research, supervised the development of the diagnostic tools and initiated most of the clinical studies.

Professor David Ray, Professor of Medicine and Endocrinology (1991-present) - evaluated the POMC and ACTH monoclonal antibodies (Abs) in defined groups of patients.

Post-Doctoral Research Associates:

Dr Steve Crosby (1988-1995) - produced monoclonal Abs to some of the POMC peptides and developed the assays

Dr Sarah Gibson (1988-1994) - produced monoclonal Abs to some of the POMC peptides and evaluated the panel of assays

Dr Alan Talbot (2000-2004) - coordinated the collection of patient samples and optimised the assay formats for development as commercial kits

Dr Lynn Pritchard (2000-2005) - evaluated the role of POMC and its constituent peptides in obesity

Dr Rachel Stovold (2009-2012) - converted the POMC assay to enable evaluation for lung cancer clinical trials (in collaboration with the Manchester Cancer Research Centre).

The aim of the research was to determine if there are diseases where the prohormone, POMC, was not processed to the active hormone, ACTH. Measurement of POMC could then be used in diagnosis and understanding the clinical symptoms and/or monitoring the disease state. This was achieved via the following steps:

i. Produced monoclonal Abs that bind to key sequences within the large POMC peptide, then selected pairs of these Abs to develop very specific and sensitive assays for the prohormone and the other hormones present within the sequence [1].

ii. Developed and characterised diagnostic assays that could be used for measurement of POMC and related peptides in blood [1].

iii. Proved that a subset of hormone-secreting tumours secrete POMC into the bloodstream using the diagnostic assay [2,3].

iv. Utilised these sensitive and specific assays for POMC and the associated ACTH-related peptides to investigate the role of POMC in hormone-secreting tumours [2,3], and in energy balance and obesity [4,5].

v. Determined that POMC is present in the circulation of normal subjects but is regulated differently to the peptides produced from it (e.g. ACTH) [6]. This is important in the POMC/ACTH/cortisol response to stress and could have implications for a personalised medicine approach to treating type 2 diabetes.

3. References to the research

The research was published in leading journals in the endocrinology field. Key papers appeared in 1994 and there have been over 40 papers substantiating the impact to date.

1. **Gibson S, Crosby SR**, Stewart MF, McCall E & **White A** (1994). Differential release of pro-opiomelanocortin-derived peptides from the human pituitary: evidence from a panel of two-site immunoradiometric assays. *J. Clin. Endocrinol. Metab.* 78, 835-841. DOI: 10.1210/jc.78.4.835
2. Stewart PM, **Gibson S, Crosby SR**, Penn R, Holder R, Ferry D, Thatcher N, Phillips P, London DR & **White A**. (1994). ACTH Precursors characterize the ectopic ACTH syndrome. *Clin. Endocrinol.* 40,199-204. DOI: 10.1111/j.1365-2265.1994.tb02468.x
3. **White A**, **Ray DW**, **Talbot A**, Abraham P, Thody AJ, and Bevan JS (2000). Cushing's syndrome due to phaeochromocytoma secreting the precursors of adrenocorticotropin. *J. Clin. Endocrinol. Metab.* 85, 4771-4775. DOI: 10.1210/jc.85.12.4771
4. Challis BG, **Pritchard LE**, Creemers JWM, Keogh JM, Yeo GSH, Bhattacharyya S, **White A**, Farooqui IS, & O'Rahilly S (2002). A missense mutation disrupting a dibasic prohormone processing site in pro-opiomelanocortin (POMC) increases susceptibility to early-onset obesity through a novel molecular mechanism. *Hum. Mol. Genet.* 11, 17, 1997-2004. DOI:10.1093/hmg/11.17.1997
5. **Pritchard LE**, Oliver RL, McLoughlin JD, Birtles S, Lawrence CB, Turnbull AV & **White A** (2003). Pro-opiomelanocortin-derived peptides in rat cerebrospinal fluid and hypothalamic extracts: evidence that secretion is regulated with respect to energy balance. *Endocrinol.* 144 (3): 760-766. DOI:10.1210/en.2002-220866
6. Russell GM, Henley DE, Leendertz J, Douthwaite JA, Wood SA, Stevens A, Woltersdorf WW, Peters BWMM, Ruigt Ge SF, **White A**, Veldhuis JD, Lightman SL (2010). Rapid glucocorticoid receptor-mediated inhibition of hypothalamic-pituitary-adrenal ultradian activity in healthy males. *J. Neurosci.*, 30: 6106-6115. DOI:10.1523/JNEUROSCI.5332-09.2010

4. Details of the impact

Context

Measurement of stress hormones in blood and other tissues has provided a step-change in the assessment of life-threatening endocrine and metabolic disorders and has underpinned the advance in knowledge of many physiological processes. However understanding the hormones involved in the body's response to stress and the effects on metabolism lagged behind, and this has limited studies on hormone secreting tumours, and the implications of hormone interactions in progression of Type 2 diabetes and mechanisms resulting in obesity.

The limitations in this field were in part because the techniques to measure ACTH were problematical. White's approach to produce monoclonal Abs to ACTH enabled them to develop a "new generation" immunoradiometric assay for quantifying ACTH. This was the first such assay and the Abs were licensed to a number of companies to enable them to develop diagnostic assays. It also provided proof of concept to allow other companies to develop diagnostic assays for ACTH.

White's research then contributed significantly to the discovery that the precursor of ACTH (POMC) was released into the blood in addition to ACTH [2]. Her research group designed and produced monoclonal Abs to POMC and developed the first immunoradiometric assay. This provided evidence that ACTH assays are also measuring ACTH precursors but to varying degrees depending on the assay (1-10%). Patients with non-pituitary tumours causing Cushing's syndrome and those with pituitary macroadenomas can have much higher concentrations of POMC than ACTH. This can be diagnosed using the POMC assay but ACTH assays may mis-represent the underlying pathology. This diagnostic tool has been used to support clinicians in the UK and internationally in the diagnosis and treatment of patients with ACTH-related disorders.

Pathways to impact

The research was presented at leading conferences (e.g. American Endocrine Society Meeting) and published in leading endocrine journals (e.g. Journal of Clinical Endocrinology and Metabolism

[e.g. 1,2,3]). This led to the POMC Abs being licensed to Immunodiagnostic Systems (IDS) in 1998, who developed the POMC assay into a kit which was available to clinical endocrinologists and researchers worldwide. The discovery that POMC was released into the blood by specific lung tumours [2] led to a grant from CRUK in 2009, and the exclusive licence for the POMC assay was transferred from IDS to CRUK. The understanding of the cross-reactivity of POMC in the ACTH assay [1,2,3], led Roche Diagnostics to take a non-exclusive licence agreement in 2004 and pay £200k for the Abs. With collaboration from White's research team, Roche incorporated the ACTH monoclonal Abs into diagnostic testing kits, which is sold and used in hospital labs worldwide.

Technologies for measuring POMC, ACTH and other POMC-derived peptides have continued to be developed using White's Abs and assay formats. Under a Material Transfer Agreement set up in November 2012, IDS are incorporating the Abs to ACTH into an automated analyser system. Once the assay has been fully characterised, a licence agreement will be set up and it is anticipated that the assay will be in the analyser and used in hospitals for diagnosis in autumn 2013. POMC (provided by White) is now being included in the National External Quality Assessment Service for all ACTH assays in the UK.

Reach and significance of the impact

White's Abs and assays for POMC and other ACTH-related peptides are used and sold worldwide for diagnosis, decisions on treatment, monitoring for recurrence of tumours and prognosis in a number of patient groups with endocrine disorders. This therefore has global health impact in relieving suffering and in improving patient care, as well as commercial impact in sales of ACTH assays and analysers.

Commercial impact:

Roche Diagnostics – sales of ACTH tests: The Abs to ACTH have been incorporated into diagnostic testing kits by Roche ("ACTH Elecsys" tests), which have been sold in all regions worldwide (e.g. UK, UAE, Brazil, Russia, Thailand and South Africa) [A]. Roche confirm that "the ACTH Elecsys tests are an important part of their portfolio and more than 6 million tests were sold from 2008-2013" [A]. Roche is the world market leader in in-vitro diagnostics and has 16% market share.

AstraZeneca – investment in research and influencing company strategy: Measurement of POMC and ACTH in human blood is also valuable to assess the stress hormone axis and is predicted to underpin the personalized medicine approach to treatment of type 2 diabetes. White has a collaboration with AstraZeneca (AZ) to measure these hormones, which were part of their drug discovery programme for type 2 diabetes/obesity and subsequently for other indications [B]. This was underpinned by £578k total funding and £1.5m contribution of resources and reagents at AZ (2008-2014), and joint publications (including [5] and [C]). Project Leader for the Cardiovascular and Metabolic Diseases therapy area at AZ states that his collaboration with White's group has been "critical to our understanding of potential drug targets... and the assay systems used to drive these drug discovery programs" and "instrumental in AZ's planned translational strategies for evaluating biomarkers of HPA axis activation", including its "decision not to progress its clinical evaluation of 11betaHSD1 inhibitors..." [B].

Impact on health – improved diagnosis and management of disease:

Endocrine disorders*:* White collaborates with physicians worldwide in assessing patients with abnormalities in secretion of POMC and other ACTH-related hormones, disorders with significant morbidity and mortality. Head of the Endocrine Consult Service at the NIH Clinical Center states: "the work of Dr. White's laboratory has had substantial public health ramifications and is likely to improve our ability to make the proper diagnosis in adrenal disorders" [D]. A leading expert on diagnostic assays for hormones in the USA at the Medical College of Wisconsin, states: "... the work from Dr. White's laboratory has revolutionized how these patients are evaluated" and "... is a paradigm for truly translational research where a brilliant basic researcher works closely with clinical colleagues to greatly advance and improve the tools we use for the laboratory diagnosis of pituitary-adrenal disorders" [E].

A Professor of Medicine at Columbia University has developed a POMC assay using White's Abs, which she uses to advance the clinical care of patients with neuroendocrine diseases (including Cushing's Syndrome) and her research on the brain regulation of energy balance in humans [F].

Although these endocrine conditions are relatively rare (e.g. ~500 pa in the USA), they are often complex and an improved understanding of the case has marked benefits. Where the patient has a complicated aetiology, White's research group undertakes more detailed analysis to find the underlying cause of their clinical symptoms (10-20 patients per year). Her research informs these investigations and the diagnostic assays they undertake provide valuable information for the treatment of these patients. White's work is cited in the global clinical decision support resource UpToDate ("The only clinical decision support resource associated with improved patient outcomes") in the article "*Measurement of ACTH, CRH, and other hypothalamic and pituitary peptides"* [G].

Cancer*:* Using the assays, White discovered that POMC is released into the blood by ectopic tumours (which are outside the pituitary gland), affecting ~10-20 patients pa in the UK, whereas tumours in the pituitary primarily release ACTH [2], thus allowing these two types of tumours to be distinguished. Following this discovery, a leading expert on diagnostic assays for hormones in the USA uses the Abs to evaluate such patients ("these assays have greatly improved the ability to diagnose and treat.." these patients – [E]), and White's research group has identified POMC in a subset of patients with small cell lung cancer as a potential biomarker of disease progression, funded by a grant from CRUK (2009-11, £111k) (Stovold *et al*., Br J Cancer 2013, doi: 10.1038/bjc.2013.112).

Obesity*:* A number of children with profound obesity have mutations in neuropeptide pathways in the brain. In four patients, measurement of POMC using White's assays has been important in the elucidation of abnormalities in the processing of POMC in the hypothalamus where it regulates food intake [4,5]. A Professor of Clinical Biochemistry & Medicine at the University of Cambridge has reported that "this has facilitated the identification of two previously unknown human syndromes", and "making these diagnoses has aided patient counselling, prognostication and is now leading to plans for trials of targeted therapy".

5. Sources to corroborate the impact

A. Supporting statement from International Business Leader for Endocrinology, Anemia & Thyroid, and Senior International Product Manager for HbA1c, Insulin, Cortisol, ACTH, & PCCC, Roche Diagnostics International Ltd. providing evidence of worldwide sales of the ACTH diagnostic testing kits incorporating White's ACTH antibodies.

B. Supporting statement from Senior Director for Cardiovascular and Metabolic Diseases at AstraZeneca, describing the value of using the assays in AZ's pre-clinical discovery programme.

C. Joint publication with AZ demonstrating White's contribution to the preclinical work-up of compounds for diabetes/obesity: Harno, E., Cottrell, EC., Yu, A., DeSchoolmeester, J., Morentin Gutierrez, P., Denn, M., Swales, JG., Goldberg, FW., Bohlooly-Y, M., Andersén, H., Wild, MJ., Turnbull, AV., Leighton, B., White, A. 11β-HSD1 inhibitors still improve metabolic phenotype in male 11β-HSD1 knock-out mice suggesting off-target mechanisms. *Endocrinology. 2013 Oct 29. [Epub ahead of print]*.

D. Supporting statement from Head of the Endocrine Consult Service at the NIH Clinical Center - leading authority on patients with ACTH-related disorders.

E. Supporting statement from a leading expert on diagnostic assays for hormones in the USA (Professor of Medicine and Director of Endocrine Research Laboratory, Medical College of Wisconsin) – evidence that the novel assay kits are of value for diagnosis of endocrine disorders in patients in the US.

F. Supporting statement from Professor of Medicine, Columbia University, New York, and Attending Physician, New York Presbyterian Hospital – uses the POMC assay to advance the clinical care of patients with neuroendocrine diseases.

G. White's work is cited in the global clinical decision support resource UpToDate: "*Measurement of ACTH, CRH, and other hypothalamic and pituitary peptides"* - http://www.uptodate.com/contents/measurement-of-acth-crh-and-other-hypothalamic-and-pituitary-peptides